

Key Factor <sup>a,b</sup>	Type of evidence to consider	Data	Evidence for stronger relevance?	Evidence for weaker weight of association?	Comments or Null Evidence
Temporality	Timing of exposures and response	Tox	<p>Studies in various species in which TCE (or metabolites DCA or TCA) were administered during a sensitive period of in utero cardiac development resulted in morphological and/or functional alterations.</p> <ul style="list-style-type: none"> <li>Drinking water administration of TCE to rats on GD 1-22 resulted in a statistically significant treatment-related increase in the incidence of cardiac defects (<u>Dawson et al., 1993; Johnson et al., 2003</u>).</li> <li>Drinking water administration of TCA (the TCE oxidative metabolite) to rats on GD 1-22 resulted in a statistically significant treatment-related increase in the incidence of cardiac defects (<u>Johnson et al., 1998a</u>). Gavage administration of TCE metabolites (DCA and TCA) on GD 6-15 (<u>Smith et al., 1989, 1992</u>) or of DCA during discrete windows of time within GD 6-15 (<u>Epstein et al., 1992</u>) resulted in treatment-related increases in the incidences of cardiac defects.</li> <li>Avian <i>in ovo</i> studies that administered TCE or TCA during the period of valvuloseptal morphogenesis (e.g., HH 15-20) resulted in altered cardiac morphology and/or function (<u>Drake, V. et al., 2006; Drake, V. J. et al., 2006; Loeber et al., 1988; Rufer et al., 2010</u>).</li> <li>A study of DCA exposure to zebra fish (<u>Hassoun et al., 2005</u>) demonstrated</li> </ul>	<p>Some in vivo or <i>in vitro</i> studies rodent studies in which TCE (or metabolites DCA or TCA) was administered during a sensitive period of in utero cardiac development resulted in no morphological alterations.</p> <ul style="list-style-type: none"> <li>Gavage administration of TCE or metabolites (DCA and TCA) to rats on GD 6-15 did not result in treatment-related cardiac defects (<u>Fisher et al., 2001</u>).</li> <li>Inhalation exposures of TCE to rats on GD 6-20 (<u>Carney et al., 2006</u>) or to rats and mice on GD 6-15 (<u>Schwetz et al., 1975</u>) did not result in treatment-related cardiac defects.</li> </ul>	

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			evidence of a disruption in cardiac development (pericardial edema and altered heart rate).	<ul style="list-style-type: none"> <li>Mouse whole embryo culture studies of DCA and TCA administered at the period of 3-6 somites detected cardiac defects (Hunter et al., 1996); a chicken whole embryo culture study of TCE administered at HH 13-14 detected alterations in AV cushion (Mishima et al., 2006).</li> <li>Avian atrioventricular canal cell culture (HH 16) study found evidence of inhibited endothelial cell separation and early events of mesenchymal cell formation in the heart following TCE exposures (Boyer et al., 2000).</li> </ul>	
Strength of association	Study quality, including study strengths and limitations	Tox	<p>Exposure occurs before outcomes onset</p> <p>Epi</p> <ul style="list-style-type: none"> <li>Four cohort or case-control studies consider temporality (Forand et al., 2012; Goldberg et al., 1990; Ruckart et al., 2013; Yauck et al., 2004). Three studies observe an association between the TCE exposure surrogate and major cardiac defects (Forand et al., 2012; Goldberg et al., 1990; Yauck et al., 2004). An association with conotruncal defects, specifically, observed in Forand et al. (2012).</li> </ul>	<ul style="list-style-type: none"> <li>Temporality was not considered in Bove (1996); Bove et al. (1995); Goldberg et al. (1990); and Lagakos et al. (1986)</li> </ul>	<ul style="list-style-type: none"> <li>The small numbers of conotruncal heart defects in Ruckart et al. (2013) precluded any analysis of this endpoint and TCE exposure.</li> </ul>
			<ul style="list-style-type: none"> <li>For Dawson et al. (1993); Johnson et al. (1998a); and Johnson et al. (2003), all of which detected cardiac malformations, study quality strengths include randomized assignment to test group, detailed description of</li> </ul>	<ul style="list-style-type: none"> <li>Johnson et al. (2003) major study quality limitations include the use of data pooled from separate study cohorts conducted over an approximately 6-year period, the use of tap water as the vehicle for some of</li> </ul>	<ul style="list-style-type: none"> <li>Some studies that reported no cardiac defects following TCE gestational exposures (Hardin et al., 1981; Healy et al., 1982; Narotsky et al., 1995;</li> </ul>

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			<p>fetal cardiac dissection and evaluation procedures, evaluation of fetal hearts without knowledge of treatment group, and confirmation of all cardiac defects by consensus of 3 experts.</p> <p>Statistical analysis of data from this study was appropriately conducted by EPA statisticians using individual fetal and litter data that were provided by the study author.</p> <ul style="list-style-type: none"> <li>• The power of detection in the <u>Johnson et al. (2003)</u> study was enhanced by the use of historical controls that did not demonstrate a temporal shift in cardiac defects. A significant dose related trend in cardiac defects was observed even without large group sizes.</li> <li>• A strong association of exposure to response was observed at high dose levels in multiple studies that identified cardiac defects. In <u>Johnson et al. (2003)</u> there was a highly significant positive trend for cardiac defects.</li> </ul> <p>Potential confounding factors exist in studies that did not identify cardiac defects (e.g., different routes of exposure, the use of different rodent strains or suppliers across studies, and the use of soybean oil as a vehicle in (<u>Fisher et al., 2001</u>).</p>	<p>control and treated groups (as reported by <u>Dawson et al. (1993)</u> with no characterization of possible contaminants and incomplete reporting of study methods and results.</p> <ul style="list-style-type: none"> <li>• While <u>Dawson et al. (1993)</u> indicated that levels of TCE in dose formulations were tested by gas chromatography, the analytical findings were not reported. <u>Johnson et al. (2003)</u> did not report whether dose formulations were analyzed. Further, levels of TCE were not assessed in the vehicle control water; therefore, it is plausible that TCE contaminated the water and that doses were actually higher than measured.</li> <li>• The <u>Dawson et al. (1993)</u> and <u>Johnson et al. (2003)</u> studies estimated doses based on the average water consumption. This method does not provide precise information to calculate TCE dose because variability in drinking water consumption among dams is not characterized.</li> <li>• The dose selection for <u>Johnson et al. (2003)</u> resulted in a NOAEL that is approximately 700-fold lower than the next highest dose.</li> <li>• Some studies that did not identify treatment-related cardiac defects following developmental exposures to TCE (e.g., <u>Carney et al., 2006</u>; <u>Fisher et</u></li> </ul>	<p><u>Narotsky and Kavlock, 1995</u>) or avian in ovo studies (<u>Bross et al., 1983; Elovaara et al., 1979</u>) did not indicate that detailed evaluation of fetal hearts was conducted.</p> <ul style="list-style-type: none"> <li>• A rat whole embryo culture study of TCE administered at the period of 4-7 somites detected no cardiac defects in a study by <u>Sailenfant et al. (1995)</u>; however, the study methods indicate that there was no evaluation of the embryonic heart.</li> </ul>

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			<ul style="list-style-type: none"> <li>• One study (<a href="#">Fisher et al., 2001</a>) attempted to replicate the methods used in the <a href="#">Johnson et al. (2003)</a> study, utilizing the same fetal cardiac dissection and evaluation techniques, and including one of <a href="#">Johnson et al. (2003)</a> study authors in the assessment team, yet found no treatment-related cardiac defects.</li> <li>• No association in <a href="#">Yauck et al. (2004)</a> in mothers &lt;38 years of age and maternal residence within 1.32 miles from at least one TCE emissions source nor in <a href="#">Lagakos et al. (1986)</a>, which does not observe an association with cardiac defects. Alternative reasons such as lower statistical power may explain these observations.</li> </ul> <p>{ 2012). A fourth study observed an increased risk estimate of 6.2 (95% CI: 2.6, 14.5) for cardiac defects in infants of mothers aged &gt;38 years and maternal residence within 1.32 miles from at least one TCE emissions source (<a href="#">Yauck et al., 2004</a>).</p>	<p><a href="#">al., 2001; Schwetz et al., 1975</a>) were well-conducted and adequately-reported GLP and/or guideline studies with no substantive limitations identified.</p> <p>• One study (<a href="#">Fisher et al., 2001</a>) attempted to replicate the methods used in the <a href="#">Johnson et al. (2003)</a> study, utilizing the same fetal cardiac dissection and evaluation techniques, and including one of <a href="#">Johnson et al. (2003)</a> study authors in the assessment team, yet found no treatment-related cardiac defects.</p> <p>• No association in <a href="#">Yauck et al. (2004)</a> in mothers &lt;38 years of age and maternal residence within 1.32 miles from at least one TCE emissions source nor in <a href="#">Lagakos et al. (1986)</a>, which does not observe an association with cardiac defects. Alternative reasons such as lower statistical power may explain these observations.</p> <p>{ 2012). A fourth study observed an increased risk estimate of 6.2 (95% CI: 2.6, 14.5) for cardiac defects in infants of mothers aged &gt;38 years and maternal residence within 1.32 miles from at least one TCE emissions source (<a href="#">Yauck et al., 2004</a>).</p>	

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Variability analysis	Sources of within- and cross-study variability that contribute to uncertainty	Tox	<ul style="list-style-type: none"> <li>Johnson et al. (2003) test subject source, husbandry, and randomization procedures were consistent across all cohorts, i.e., including Dawson et al. (1993) and metabolite studies Johnson et al. (2003). Fetal cardiac evaluation methodology, which included evaluation without knowledge of treatment group and confirmation of all cardiac anomalies by 3 expert scientists, was also consistently applied across cohorts and studies from the UAZ laboratory. This had the result of reducing intra- and inter-study variability in the assessment.</li> </ul>	<ul style="list-style-type: none"> <li>Johnson et al. (2003) reported that cardiac defect incidences were consistent across all control cohorts (55 litters over approximately 6 years). An EPA review of the available control data did not observe unusual heterogeneity in prevalence of malformations.</li> <li>Studies that reported cardiac defects following administration of metabolites (DCA and TCA) used randomized assignment of maternal animals to test group, thus reducing intra-study variability.</li> <li>Although Dawson et al. (1993) and Johnson et al. (2003) identified cardiac defects following exposures to TCE during development, (Carney et al., 2006; Fisher et al., 2001; Schwetz et</li> </ul>	<ul style="list-style-type: none"> <li>Based upon the toxicokinetic profile of TCE (EPA, 2011e), it is considered unlikely that toxicokinetic factors contributed significantly to differences in response across study protocols.</li> <li>The Johnson et al. (2003) study reported data from several cohorts of animals, which were on study over a period of approximately 6 years. The data included control cohorts, some of which were concurrent and some that were non-concurrent to the TCE-treated groups (Johnson et al., 2005; Johnson, 2014). Data that definitively link the individual control litter response data with each particular cohort are no longer available for independent examination.</li> <li>Different study outcomes were observed in studies that had many similarities in study design and conduct, i.e., Dawson et al. (1993) and Johnson et al. (2003) identified exposure related cardiac defects while Fisher et al. (2001) did not. In the Fisher et al. (2001) study, care was taken to ensure that the same cardiac evaluation methods were used as in the Dawson et al. (1993) and Johnson et al. (2003) studies, including fetal evaluation with knowledge of treatment group, and one of the study authors of Johnson et al. (2003) participated in the fetal examination.</li> <li>The use of soy bean oil in the Fisher et al. (2001) study vs. water vehicle and control for Johnson et al. (2003) and Dawson et al. (1993) studies.</li> <li>The Johnson et al. (2003) and Dawson</li> </ul>

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			<p>al., 1975) did not find treatment-related cardiac abnormalities. This may be the result of differences in the study design and assessment methods. This includes such aspects as animal strain, age, source, exposure route and vehicle, duration of exposure, and cardiac evaluation methods.</p>	<p>et al. (1993) studies did not calculate variability in TCE dose by measuring individual dam water consumption.</p>	
<p><b>Uncertainty analysis</b></p> <p>Missing information or data gaps, within and across studies</p>	<p>Tox</p> <ul style="list-style-type: none"> <li>• NE (not considered in Hill analysis)</li> <li>• NE (not considered in Hill analysis)</li> </ul>	<p>Sources of within- and cross-study variability that contribute to uncertainty</p>	<ul style="list-style-type: none"> <li>• For the studies conducted by the UAZ laboratory that identified cardiac defects following exposures to TCE, DCA, or TCA (Dawson et al., 1993; Johnson et al., 1998a; Johnson et al., 2003), detailed descriptions of evaluation methods for assessment of cardiovascular effects were provided.</li> <li>• Individual fetal and litter cardiac findings data, as well as detailed information on study conduct and fetal evaluation methods, were provided to the EPA for Dawson et al. (1993) and Johnson et al. (2003).</li> </ul>	<ul style="list-style-type: none"> <li>• The publications for studies conducted by the UAZ laboratory that identified cardiac defects following exposures to TCE, DCA, or TCA (Dawson et al., 1993; Johnson et al., 1998a; Johnson et al., 2003) did not report essential study details, and generally did not include summaries of maternal data or fetal data for endpoints other than cardiac defects.</li> <li>• For well-conducted studies that did not detect cardiac defects following developmental exposures to TCE or metabolites (Carney et al., 2006; Fisher et al., 2001) adequate descriptions of study methodology and summary data</li> </ul>	<ul style="list-style-type: none"> <li>• Studies examined different populations, exposure levels, gradients, and media. Additionally, different sets of strengths and uncertainties in this set of studies would contribute to observed cross-study variability.</li> </ul>

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				<ul style="list-style-type: none"> <li>for maternal and fetal findings were reported.</li> <li>Mechanistic data for alterations in cardiac development are limited and do not identify initiating events for the putative AOP.</li> </ul>	
Missing information or data gaps, within and across studies	Epi	<ul style="list-style-type: none"> <li>NE (not considered in Hill analysis)</li> </ul>	<ul style="list-style-type: none"> <li>NE (not considered in Hill analysis)</li> </ul>		
<b>Qualitative dose-response</b>	Association between exposure/dose and degree of effect	Tox	<ul style="list-style-type: none"> <li>Alterations in cardiac development were observed in multiple studies at high dose levels following TCE, DCA, or TCA exposures (Dawson et al., 1993; Johnson et al., 1998a; Johnson et al., 2003; Smith et al., 1989, 1992).</li> <li>The incidence of cardiovascular effects increased as a function of dose in Johnson et al. (2003).</li> <li>An association between exposure to TCE (or DCA or TCA) and alterations in <del>cardiac development</del> was reported in various animal models, i.e., LE and SD rats, CD-1 mice, chicken embryos, and zebra fish (Dawson et al., 1993; Drake, V. et al., 2006; Drake, V. J. et al., 2006; Hassoun et al., 2005; Johnson et al., 2003; Smith et al., 1989, 1992; Williams et al., 2006).</li> </ul>	<ul style="list-style-type: none"> <li>The dose response for cardiac defects identified by Johnson et al. (2003) could only be fit to a model with elimination of the high dose data from the analysis. The lowest dose tested had a zero response for cardiac defects, below the historical control incidence. The doses tested were spaced over several orders of magnitude, with wide gaps.</li> <li>Carney et al. (2006) was the only other study in the database that evaluated developmental effects of TCE over multiple dose levels. In that study, no fetal toxicity and minimal maternal toxicity was reported.</li> <li>TCE doses tested in Dawson et al. (1993) and Johnson et al. (2003) (drinking water): 2.5 ppb, 250 ppb, 1.5 ppm, or 1100 ppm (0, 0.00045, 0.048, 0.218, or 129 mg/kg-day)</li> <li>TCE doses tested Fisher et al. (2001) (gavage): 500 mg/kg-day</li> <li>TCE doses tested in Carney et al. (2006) (inhalation): 50, 150, or 600 ppm (268.5, 805.5, or 3222 mg/m<sup>3</sup>)</li> </ul>	

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			outcome of interest was incorporated in the analysis. A significant dose-response trend was identified, whether or not the high dose value was included in the analysis.	<ul style="list-style-type: none"> <li>• NE</li> <li>• <u>Goldberg et al. (1990)</u> and <u>Lagakos et al. (1986)</u> examined exposure-response; none observed.</li> </ul>	
<b>Experimental evidence</b>	Hypothesis testing: manipulation of exposure scenario with resulting alterations in response	Tox	<ul style="list-style-type: none"> <li>• A study by <u>Epstein et al. (1992)</u> administered the metabolite DCA to rats on varied days of gestation and identified critical windows of exposure for eliciting cardiac developmental defects.</li> <li>• No statistically significant increases in congenital heart defects were observed in groups of rats that were exposed to TCE prior to pregnancy only <u>Dawson et al. (1993)</u>.</li> <li>• <u>Drake, V. J. et al. (2006)</u> demonstrated that cardiac defects did not occur in chick embryos exposed to TCE and specification (approximately GD 6 in rats) rather than the period of valvuloseptal morphogenesis.</li> </ul>	<ul style="list-style-type: none"> <li>• Studies in rodents that administered TCE via drinking water detected an increase in fetuses with cardiac defects (<u>Dawson et al., 1993</u>; <u>Johnson et al., 2003</u>); studies that administered TCE via other routes (gavage and inhalation) were negative for this response (<u>Carney et al., 2006</u>; <u>Fisher et al., 2001</u>; <u>Schwartz et al., 1975</u>).</li> <li>• In a whole embryo culture (WEC) study of DCA and TCA (<u>Hunter et al., 1996</u>), that identified cardiac defects, the acid nature of DCA and TCA may have impacted dysmorphogenesis.</li> </ul>	

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Association not observed once exposure ceases	Epi	• NE	No differences between observed and expected numbers of cardiac defect cases once wells were closed in contaminated area (Goldberg et al., 1990).		
<b>Reproducibility [Consistency]</b>	Reproducibility: Corroboration across studies, labs, routes of exposure, species, etc.	Tox	<ul style="list-style-type: none"> <li>Studies that administered TCE in drinking water to rats on GD 1-22 were conducted over a period of approximately 6 years by researchers at the same academic facility (UAZ, Tucson) used the same cardiac evaluation methods and identified treatment and dose-related cardiac malformations (Dawson et al., 1993; Johnson et al., 1998a; Johnson et al., 2003). A preliminary screening study that utilized intrauterine administration of TCE also detected cardiac defects (Dawson et al., 1990).</li> <li>The types of cardiac malformations observed were similar across study cohorts and treatment groups throughout the duration of the research program.</li> <li>Studies on TCE metabolites (TCA and TCA) conducted in other laboratories (Epstein et al., 1992; Smith et al., 1989, 1992) identified cardiac defects similar to those observed in the UAZ studies.</li> <li>Cardiac septal anomalies were observed in avian <i>in ovo</i> studies (Drake, V. et al., 2006; Rufer et al., 2010), and in WEC assays (Hunter et</li> </ul>	<ul style="list-style-type: none"> <li>Studies conducted in other laboratories than UAZ and that administered TCE by gavage or inhalation (Carney et al., 2006; Fisher et al., 2001; Schwetz et al., 1975) did not identify statistically significant increases in cardiac defects. Fisher et al. (2001) used the same cardiac evaluation methods as the UAZ lab.</li> <li>Studies that did not identify cardiac defects with TCE and/or metabolite exposures (Carney et al., 2006; Fisher et al., 2001; Schwetz et al., 1975) did not replicate all aspects of the Johnson et al. (2003) study, even though Fisher et al. (2001) used the same cardiac evaluation techniques as Johnson et al. (2003) and Dawson et al. (1993), and therefore provide only limited evidence of lack of reproducibility.</li> </ul>	

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			<p>al., 1996; Mishima et al., 2006) with TCE and/or metabolite exposures.</p> <p>Zebrafish studies also demonstrated evidence of alterations in cardiac development (Hassoun et al., 2005; Williams et al., 2006).</p>	<ul style="list-style-type: none"> <li>Association between cardiac defects and TCE exposure surrogate observed in four studies. These studies were of different populations living in different places, time and circumstances.</li> </ul> <p>Lagakos et al. (1986) compared a pregnancy receiving contaminated residential well water to a pregnancy not receiving residential water from contaminated wells and does not observe an association between cardiac defects and contaminated drinking water.</p>	
<b>Biological plausibility</b>	Observed outcome can be attributed to toxic insult given the known science	Tox	<ul style="list-style-type: none"> <li>Avian in ovo studies and atrioventricular cell culture studies support the biological plausibility of effects of TCE on cardiac development, given that early chick heart development is similar to mammalian (including human), particularly regarding the role of the cardiac cushion in septation (NRC, 2006; Richards and Garg, 2010).</li> <li>Preliminary exploration of a possible adverse outcome pathway (AOP) has resulted in a reasonable conceptual</li> </ul>	<ul style="list-style-type: none"> <li>A definitive AOP for TCE-induced cardiac defects, including a putative initiating event, has not yet been characterized. Additional mechanistic data are needed to support the hypothesized AOP.</li> <li>There are insufficient mechanistic data to characterize additional potential MOAs other than that hypothesized in the AOP.</li> </ul>	<ul style="list-style-type: none"> <li>It is possible that multiple modes of action are involved in alterations to cardiac development.</li> </ul>

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			model for TCE-induced congenital heart defects. In this construct, the vulnerable period is defined by endocardial morphogenesis.  Endothelial-mesenchymal transition is disrupted in the area of the atrioventricular canal, leading to septal defects. Possible genetic contributions to abnormal cardiac development include disruption of TGF-beta pathway, endrin pathway, Notch pathway, VEGF pathway, and RXR signaling. At a cellular level, epithelial-mesenchymal transition may be affected in the endocardium, at the tissue level, there is altered cellularity of the endocardial cushion, and secondary effects such as dysregulation of cellular Ca <sup>2+</sup> fluxes may result in additional impacts on the developing heart.	• NE	• In vitro and in vivo animal studies report cardiac defects with TCE and TCE-metabolite exposure.
Observed association plausible given the known science	Epi			• NE	
Alternative or multiple explanations	Other possible explanations for observed outcome after the exposure of interest	Tox	Given the presumed contribution of both environmental exposures and genetic predisposition in human congenital heart disease ( <u>Richards and Garg, 2010</u> ), it is possible that the test subjects used in the <u>Johnson et al. (2003)</u> study and others conducted in that laboratory may have been	• There is a possibility that cardiac defects detected in the <u>Dawson et al. (1993)</u> study were associated in part with the use of tap water as a control vehicle (i.e., possible presence of contaminants).	

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			<p>particularly susceptible to alterations in cardiac development.</p> <ul style="list-style-type: none"> <li>Other contributing factors or confounding factors were not specifically identified in the evaluated in-vivo studies.</li> <li>It is possible that the absence of treatment-related cardiac defects in well-conducted TCE studies (<u>Carney et al., 2006; Fisher et al., 2001</u>) or metabolite studies (<u>Fisher et al., 2001</u>) was due to confounding variables such as differences in strain/source of animal model, route of exposure, toxicokinetics, vehicle [e.g., soybean oil in <u>Fisher et al. (2001)</u>], or differences in cardiac evaluation methods.</li> <li>It is unlikely that the cardiac defects observed by <u>Johnson et al. (2003)</u> were an artifact of the evaluation procedures used, since a study by <u>Fisher et al. (2001)</u>, using the same fetal cardiac evaluation procedures, did not identify an association between TCE exposure and the incidence of cardiac defects.</li> </ul>		
	Other possible explanations for observed outcome after the exposure of interest (not considered in Bove et al., 1995).	Epi	<ul style="list-style-type: none"> <li>Potential maternal risk factors were adjusted in statistical analysis in <u>Forand et al. (2012)</u> and <u>Yauck et al. (2004)</u> or were not found in statistical analyses to influence observed association by +15% (<u>Bove, 1996</u>; <u>Bove et al., 1995</u>).</li> </ul>	<ul style="list-style-type: none"> <li>Potential for confounding from another exposure given the poor exposure definition in <u>Yauck et al. (2004)</u>. The positive association in <u>Goldberg et al. (1990)</u> may result from likely selection biases in controls.</li> </ul>	

Key Factor <sup>a,b</sup>	Type of evidence to consider	Data resulting from exposure to test substance	Evidence for stronger weight of association	Evidence for weaker weight of association	Comments or Null Evidence
Hill analysis)	Single cause and effect relationship resulting from exposure to test substance	Tox	<ul style="list-style-type: none"> <li>Cardiac defects in rats appear to be attributable to direct chemical exposure to TCE or metabolites (DCA or TCA) and are unlikely to be the result of secondary effect of maternal toxicity. <u>Johnson et al. (2003)</u> reported that TCE exposure via drinking water to pregnant rats did not result in maternal toxicity. <u>Carney et al. (2006)</u> reported minimal decreases in body weight gain in dams, with no adverse fetal outcomes. In fetuses, there was no indication of TCE-related fetal weight deficits, external or skeletal anomalies, or of soft tissue alterations other than cardiac defects in <u>Johnson et al. (2003)</u> nor in any other study.</li> </ul>	<ul style="list-style-type: none"> <li>Studies conducted in other laboratories than UAZ and that administered TCE by gavage or inhalation (<u>Carney et al., 2006; Fisher et al., 2001; Schwetz et al., 1975</u>) did not identify cardiac defects. <u>Fisher et al. (2001)</u> used the same cardiac evaluation methods as the UAZ lab. The cardiac defects detected in the <u>Dawson et al. (1993)</u> study may have been related to the use of tap water as a vehicle (i.e., possible contaminants).</li> <li>The majority of the cardiac malformations following TCE exposures to rats (<u>Dawson et al., 1993; Johnson et al., 2003</u>) or chicks (<u>Drake, V. et al., 2006; Rufier et al., 2010</u>) during sensitive periods of cardiac development were ventricular septal defects, valve defects, or outflow tract abnormalities. Mechanistic data suggest a common etiology (disruption of the cardiac cushion formation) for the observed cardiac defects <u>Boyer et al. (2000)</u>.</li> </ul> <p><i>An oral dose of TCE CVDs True for ? population</i></p>	
Specificity					

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Coherence	Single cause and effect relationship resulting from exposure to test substance	Epi	• NE	<ul style="list-style-type: none"> <li>Specificity not a critical compared to other Hill aspects since outcomes may have several risk factors. Maternal risk factors, specifically chemical risk factors, associated with cardiac defects in infants have not been well studied.</li> </ul>	
	Summary: Extent to which data are similar in outcome and exposure across database	Tox	<ul style="list-style-type: none"> <li>Multiple studies were conducted at UAZ (Dawson et al., 1993; Johnson et al., 1998a; Johnson et al., 2003), in which rats were administered TCE or metabolites DCA or TCA in drinking water on GD 1-22 and for which study design and cardiac evaluation methodologies were consistent. The outcomes of these studies (detection of cardiac defects, particularly septal defects, valve abnormalities, and outflow tract anomalies) are consistent across these studies. Additionally, these outcomes are supported by the results of avian in ovo and in vitro studies, studies with TCE metabolites (DCA and TCA) in rodents, in vitro whole embryo culture studies, and mechanistic data.</li> </ul>	<ul style="list-style-type: none"> <li>Developmental toxicity studies with TCE that were conducted in other laboratories (Carney et al., 2006; Fisher et al., 2001; Schwetz et al., 1975) administered TCE to rats of other strains or sources, using different routes of exposure (inhalation or gavage), administered on different days of gestation (i.e., not including GD 1-6) than the UAZ studies and did not identify cardiac defects. No other study in the TCE database reported cardiac defects at the low dose levels reported by Johnson et al. (2003).</li> </ul>	
	Cause and effect interpretation should not conflict with the generally known facts of the natural	Epi	<ul style="list-style-type: none"> <li>Associations in epidemiologic studies of cardiac defects and maternal occupational exposure to degreasing solvents or to organic solvents (Gilboa et al., 2012; Loffredo et al., 1991; Tikkainen and Heinonen, 1988, 1991).</li> </ul>	• NE	

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	history and biology of the disease				
NE = No relevant evidence.					

HH = Hamburger-Hamilton stages of chick development (Hamburger and Hamilton, 1951).

Tox = Animal toxicology studies; Epi = Epidemiological studies

Key Factor references:

a EPA (2006b)

b Hill (1965)



## **Appendix N      Weight-of-Evidence Analysis for Fetal Cardiac Malformations Following TCE Exposure**

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Appendix N contains a weight-of-evidence analysis for the association between short-term exposure to TCE and fetal cardiac defects.

The analysis only addresses the fetal cardiac defects observed following gestational exposures to TCE and/or its oxidative metabolites dichloroacetic acid (DCA) and trichloroacetic acid (TCA), and includes updated information that was not part of the 2011 TCE IRIS assessment. This update includes 1) identification of any new literature, 2) a systematic evaluation of available data, 3) an evaluation of the weight of evidence for the association of TCE exposures with cardiac defects, and 4) a transparent presentation of the evaluation.

A systematic literature search was conducted to identify all studies published subsequent to the final literature search that had been conducted by EPA during completion of the 2011 TCE IRIS assessment ([EPA, 2011e](#)). A total of 1686 unique citations were initially identified from PubMed, Toxline, and Web of Science (WoS). These citations were screened using the title, abstract, and/or full text for pertinence to evaluation of the developmental toxicity of TCE, TCA, and DCA exposure. The literature search identified no new animal toxicology studies of fetal cardiac defects, one new epidemiology study that assessed the association of TCE or chlorinated solvent exposures with cardiac defects, and two studies that provided mechanistic information relevant to alterations of cardiac development following TCE (or metabolite) exposures.

The analysis does not provide an update on other developmental effects of TCE exposure, i.e., ocular malformations, developmental neurotoxicity, and developmental immunotoxicity.



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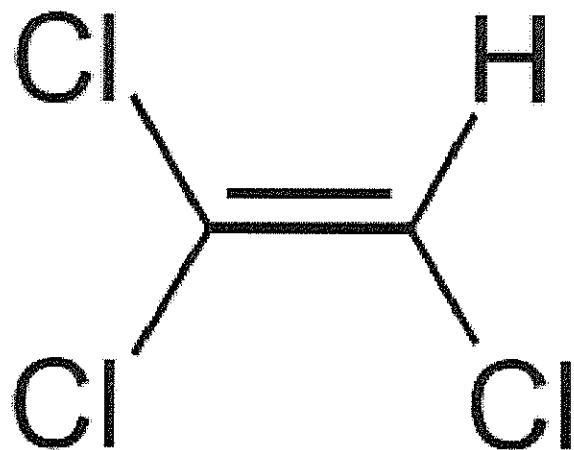
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Pollution Prevention

## TSCA Work Plan Chemical Risk Assessment

### Trichloroethylene: Degreasing, Spot Cleaning and Arts & Crafts Uses

CASRN: 79-01-6



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